

Relative Toxicity of Gossypol Enantiomers in Broilers^{1,2}

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ABSTRACT Use of cottonseed meal in poultry diets has been avoided in large part because of fear of gossypol toxicity. Gossypol exists naturally as a mixture of 2 enantiomers that exhibit different biological activities. Two experiments were conducted to determine the relative toxicity of gossypol enantiomers on broilers. In the first experiment, 3-d-old broilers were fed a standard diet containing 0, 100, 200, 300, or 400 mg of gossypol from gossypol acetic acid per kilogram of diet from 3 to 42 d of age. This form of gossypol contains both enantiomers in an equimolar ratio. Each dietary treatment consisted of 6 replicate pens of 4 birds. In the second experiment, 3-d-old broilers were divided into 15 pens of 4 birds each and fed a standard diet supplemented with either no gossypol or one of the gossypol enantiomers at 200 or 400 mg/kg of diet from 3 to 21 d of age. In both experiments, feed intake and BW gain were measured. In addition,

several organ and tissue samples were collected at 21 d (experiments 1 and 2) and 42 d (experiment 1) of age and analyzed for gossypol. In experiment 1, feed consumption and BW gain were reduced ($P < 0.05$) at 21 and 42 d for the birds fed the highest level of gossypol. The concentration of gossypol in the heart, kidney, and plasma were equivalent at 21 and 42 d of age. In experiment 2, total feed consumption was reduced only in birds consuming (-)-gossypol, but BW gains were lower for birds fed either enantiomer. However, (-)-gossypol was more detrimental to growth than (+)-gossypol. The liver had the highest tissue concentration of both enantiomers, and accumulation of (+)-gossypol was higher than (-)-gossypol in all tissues examined. No racemization of the enantiomers was apparent in the tissues analyzed. Our results indicated that both gossypol enantiomers were toxic to broilers but that (-)-gossypol was more harmful to efficient broiler production than (+)-gossypol.

(Key words: cottonseed meal, gossypol, gossypol enantiomer, broiler)

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INTRODUCTION

Cottonseed meal (CSM) could be an attractive alternative protein source for poultry diets, but concern over the presence of the potentially toxic agent, gossypol, has limited its use. Gossypol [1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3, 3'-dimethyl-(2, 2'-binaphthalene)-8, 8'-dicarboxaldehyde] is a polyphenolic compound located in pigment glands that are distributed throughout the cotton plant. Gossypol is composed of 2 naphthalene rings with restricted rotation around the bond connecting the rings. As a result of this restricted rotation, gossypol occurs naturally as a mixture of 2 enantiomers [(+)- and (-)-

gossypol] that differ in their optical properties (Huang et al., 1987).

Researchers have previously reported that feeding broilers diets containing high levels of gossypol results in depressed weight gain (Lillie and Bird, 1950; Milligan and Bird, 1951; Phelps, 1966; Waldroup, 1981) and poor feed efficiency (Couch et al., 1955; Heywang and Bird, 1955). Therefore, extensive research has been conducted to establish methods to decrease the negative impacts that gossypol might have on poultry production. Weight gain and feed efficiency are unaffected when broilers are fed a diet in which a portion of the dietary soybean meal is replaced with CSM made from glandless cottonseed, which has a very low concentration of gossypol (Waldroup et al., 1968). Thus, the development of strains of cotton with glandless seeds should alleviate the problems associated with feeding CSM to broilers (Ryan et al., 1986). However, gossypol serves as a natural insecticide in the cotton plant (Bottger et al., 1964), and therefore it is desir-

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Abbreviation Key: CSM = cottonseed meal; GAA = gossypol acetic acid.

able that the plant retains the ability to produce gossypol in its other tissues. At present, all the CSM produced commercially in the United States contains gossypol.

Another avenue of research to lower the impact of gossypol on poultry production has dealt with dietary iron supplementation. The presence of gossypol in poultry diets may be counteracted by addition of highly soluble iron salts that bind gossypol (Withers and Brewster, 1917; Gallup, 1928; Eagle, 1949). The gossypol-related brown-yolk discoloration of eggs produced by laying hens fed diets containing CSM can be prevented when crystalline ferrous sulfate heptahydrate is added to the diet at a 4:1 weight ratio of iron to free gossypol (Panigrahi, 1990; Panigrahi and Morris, 1991; Panigrahi and Plumb, 1996). However, iron supplementation is costly, contributes to the heavy metal content in feces, and can depress bird performance by reducing availability of dietary phosphorus (Panigrahi and Plumb, 1996).

Little research attention has focused on the relative toxicity of the individual gossypol enantiomers in chickens. Experiments conducted to determine the relative toxicity of the enantiomers have only been done with cotton cultivars that produce seeds with different proportions of (+)- and (–)-gossypol. A study conducted with broilers indicated that ground Pima (*Gossypium barbadense*) cottonseed with a higher (–) to (+) ratio of gossypol was significantly more toxic, based on feed intake and body weight gain than ground Pima cottonseed with a higher (+)-to-(–) ratio of gossypol (Gamboa, 1997). This was later confirmed when broilers fed Moco crushed cottonseed containing a high (+)- to (–)-gossypol enantiomer ratio performed better than those receiving Moco cottonseed with a lower (+)- to (–)-gossypol enantiomer ratio (Bailey et al., 2000). These results suggest that CSM containing a higher proportion of (+)-gossypol relative to (–)-gossypol would be more desirable for broiler production. Nevertheless, for a definitive determination of the relative toxicity of the 2 enantiomers, they would have to be fed individually in pure form. Therefore, in the present study, gossypol acetic acid (GAA), which contains an equal molar ratio of the enantiomers, and the individual gossypol enantiomers were fed to broilers. Because some gossypol derivatives (amine complexes) have been reported to racemize (Si et al., 1990; Fish et al., 1995), the possibility of interconversion of the enantiomers in broiler tissues was also investigated.

MATERIALS AND METHODS

Experiment 1

Two hundred 1-d-old Cobb × Cobb male broiler chicks were fed a standard corn-soybean meal based mash starter diet (Table 1) for 2 d. At 3 d of age, the chicks were weighed, and those with extreme weights were discarded. The remaining birds were assigned to 30 pens of

TABLE 1. Composition of the experimental diets (experiments 1 and 2)

Ingredient	Diet	
	Broiler starter ¹	Broiler grower ²
	— (g/100 g of diet DM) —	
Corn	56.12	60.80
Soybean meal, 48% CP	37.50	32.61
Poultry fat	3.07	3.43
Defluorinated phosphorus	1.75	1.56
Limestone	0.73	0.78
Salt	0.30	0.32
Vitamin mix ³	0.25	0.25
D,L-Methionine	0.20	0.17
Mineral mix ⁴	0.08	0.08
Calculated analysis ⁵		
ME (kcal/ kg)	3,080.00	3,150.00
CP (%)	22.50	20.50
Crude fat (%)	5.28	5.76
Crude fiber (%)	2.53	2.50
Lysine (%)	1.26	1.12
Calcium (%)	0.95	0.90
Available phosphorus (%)	0.45	0.41
Methionine (%)	0.57	0.52
TSAA (%)	0.93	0.85

¹Broiler starter diet used in experiments 1 and 2 from 0 to 3 wk of age.

²Grower diet used in experiment 1 from 3 to 6 wk of age.

³Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D₃, 110 IU; vitamin E, 1.1 IU; vitamin B₁₂, 0.001 mg; riboflavin, 0.44 mg; niacin, 4.41 mg; D-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; D-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.

⁴Mineral mix provided the following in milligrams per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; I, 1.5; and Se, 0.5.

⁵Calculated analysis was based on Dale (2001).

4 birds each such that the weight profile of each pen was similar. Chicks were wing-banded for individual identification. The 30 pens were randomly assigned to 5 dietary treatments consisting of the starter diet supplemented with 0, 100, 200, 300, or 400 mg/kg of gossypol from GAA. Throughout the experiment, chicks were given free access to water and mash experimental diets, brooded in thermostatically controlled batteries⁴ with raised wire floors, and reared on a 24-h lighting schedule. The Institutional Animal Care and Use Committee of the University of Georgia approved all animal procedures.

The GAA used in this experiment was recovered from cottonseed soapstock as previously described (Dowd and Pelitire, 2001) and was >99% pure. The weight of gossypol in GAA is 89.64%, and this percentage was used in calculating the amount of GAA to add to the experimental diets. Because GAA contains a 50:50 mixture of (+)- and (–)-gossypol, the concentrations of individual enantiomers were 0, 50, 100, 150, and 200 mg/kg of diet. Each of the diets was mixed daily to minimize the potential of gossypol binding to other feed components. To monitor gossypol binding, feed samples were collected at 0, 4, 8, and 24 h after mixing. These samples were frozen at –80°C until we determined free gossypol content.

At 21 d of age, 4 birds from 3 of the replicate pens were killed, and the liver, heart, kidney, and a portion of the

⁴Peterime Incubator Co., Gettysburg, OH.

left pectoralis major muscle were collected. The organs were pooled by pen, weighed, and immediately frozen at -80°C for future analysis of gossypol. Blood was collected from the brachial vein and placed on ice. Heparinized blood samples were then centrifuged for 10 min at $3,000 \times g$. Plasma was collected from each sample and frozen at -80°C for future gossypol analysis. The remaining birds were transferred to pens in a grower battery and fed daily a standard grower diet (Table 1) supplemented with the appropriate levels of gossypol. At 42 d of age, the liver, heart, kidney, testis, a portion of the left pectoralis major muscle, and the blood were removed from the remaining birds. Feed consumption and mortality were recorded daily, and BW was determined weekly throughout the experiment.

Experiment 2

The protocol for this experiment was similar to experiment 1, except that 15 pens of 4 birds each were assigned to 5 dietary treatments consisting of the starter diet supplemented with no gossypol or 200 or 400 mg/kg of each gossypol enantiomer. Tissue samples were collected from the broilers at 21 d of age. Individual gossypol enantiomers were prepared by crystallization as previously described (Dowd, 2003) and were at least 99.5% optically pure based on HPLC analysis. During this experiment, total water consumption for a 24-h period was determined on d 11, 16, and 20 for each pen. In addition, total bile volume and blood packed cell volume were determined for each bird at the end of the experiment. To reduce variation in amounts of bile related to feed intake, birds were fasted for 12 h before the gallbladder contents of each bird were collected with a needle and syringe. Blood was collected in 2 heparinized capillary tubes from the brachial vein of each bird. The capillary tubes were immediately centrifuged in a microcapillary centrifuge, and the percentage of packed cell volume was determined with a microcapillary reader.⁵

Bile Analyses

Bile dry matter was determined (AOAC, 1970) for each bird from duplicate samples of 100 μL each. To detect differences in bile pigment concentration, 20 μL of bile from each bird was diluted with 3,980 μL of deionized water, and the absorbance at 625 nm was determined with a DU-530 spectrophotometer.⁶ The remaining bile was stored at -80°C for subsequent gossypol determination.

Gossypol Determination

Feed samples were assessed for free gossypol content by the official method of the American Oil Chemists'

Society (AOCS, 1985). Tissues were freeze-dried for 48 h, which was sufficient to reach a constant dry weight. Total gossypol and (+)- and (-)-gossypol concentrations in tissues and feed samples were determined by HPLC as previously described (McMillan, 2000).

Statistical Analyses

Data from each experiment were subjected to ANOVA according to the general linear models procedure. Tukey's multiple-comparison procedure (SAS Institute, 2001) was used to detect significant differences among diets (experiments 1 and 2) and bird age (experiment 2). For experiment 1, regression analyses were conducted to test for linear and quadratic effects in broiler performance and tissue gossypol concentrations. The resulting regression models were then reduced using a stepwise statistical procedure to eliminate nonsignificant effects and determine appropriate linear and quadratic prediction equations (SAS Institute, 2001). All statistical procedures were done with SAS statistical software package (SAS Institute, 2001), and differences were considered significant when $P < 0.05$.

RESULTS

Experiment 1

Chicks fed the diet containing 400 mg of gossypol/kg were smaller ($P < 0.05$) at 21 and 42 d of age than chicks fed a diet containing 0, 100, or 200 mg of gossypol/kg (Table 2). At 21 and 42 d of age total feed consumption was lower ($P < 0.05$) for birds fed the diets containing 300 and 400 mg of gossypol/kg than for birds fed the diet containing no gossypol (Table 2). As the level of dietary gossypol increased, there was a linear decrease in feed intake (Table 2). None of the birds died during the experiment. The free gossypol content of each of the diets did not decrease during the 24 h between the daily preparations (data not shown).

Organ weights relative to BW were unaffected by gossypol supplementation at 21 and 42 d except for a decrease in relative heart size at 42 d for birds fed the diet with 400 mg/kg (data not shown). Both gossypol enantiomers were detected in all the tissues examined (Tables 3 and 4). Although the diets contained equal concentrations of (-)- and (+)-gossypol, all of the tissues examined contained more (+)-gossypol than (-)-gossypol (Tables 3 and 4). Except for the liver, tissue concentrations of either enantiomer did not increase from 21 d to 42 d of age (Tables 3 and 4). As dietary gossypol concentrations increased, concentrations of (-)- and (+)-gossypol at 42 d of age increased linearly in the heart, kidney, muscle, and plasma; however, in the liver, the concentration increased quadratically (Tables 3 and 4).

Experiment 2

Feeding either enantiomer depressed growth, but the highest dietary concentration of (-)-gossypol was the

⁵International Equipment Co., Needham Heights, MA.

⁶Beckman Coulter, Fullerton, CA.

TABLE 2. Body weight, feed consumption and feed conversion at 21 d and 42 d of age in broilers fed varying levels of gossypol from gossypol acetic acid (experiment 1)

Dietary gossypol	Body weight (g) ¹		Total feed consumption (g) ¹		Feed conversion rate (g/g) ¹	
	21 d	42 d	21 d	42 d	21 d	42 d
0	838 ± 10 ^a	2,545 ± 41 ^a	1,111 ± 7.8 ^a	3,967 ± 63.8 ^a	1.44 ± 0.02 ^{bc}	1.60 ± 0.02 ^a
100	829 ± 14 ^a	2,379 ± 60 ^a	1,060 ± 21.5 ^{ab}	3,630 ± 40.4 ^{ab}	1.39 ± 0.01 ^c	1.57 ± 0.02 ^a
200	807 ± 6 ^{ab}	2,313 ± 27 ^a	1,062 ± 7.6 ^{ab}	3,589 ± 93.4 ^{abc}	1.44 ± 0.01 ^{bc}	1.60 ± 0.03 ^a
300	758 ± 12 ^{bc}	2,154 ± 138 ^{ab}	1,025 ± 11.3 ^{bc}	3,290 ± 115.8 ^{bc}	1.49 ± 0.01 ^{ab}	1.58 ± 0.02 ^a
400	711 ± 17 ^c	1,921 ± 111 ^b	967 ± 20.0 ^c	3,113 ± 28.8 ^c	1.50 ± 0.01 ^a	1.69 ± 0.06 ^a
Regression analysis						
r^2	0.75	0.73	0.62	0.76	0.47	—
Coefficient						
Intercept	836.79†	2,557.17†	1,109.21†	3,927.65†	1.42†	—
Linear (gossypol)	—	-1.47†	-0.32†	-2.05†	—	—
Quadratic (gossypol)	0.00†	—	—	—	0.00†	—

^{a-c}Values without a common letter superscript differ ($P < 0.05$).

¹Values are means ± SEM per bird with 6 replicate pens of 4 birds at 21 d and 3 replicate pens of 4 birds at 42 d for each diet.

†Regression coefficient value is significant ($P < 0.05$).

TABLE 3. Concentrations at 21 d and 42 d of age of (-)-gossypol in tissues from broilers fed varying levels of gossypol from gossypol acetic acid (experiment 1)

Dietary gossypol	Liver		Heart		Kidney		Muscle		Plasma	
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
	— (µg/g of DM) ^{1,2} —									
100	61 ± 2.9 ^c	82 ± 10.9 ^{c*}	9 ± 1.2 ^b	18 ± 4.3 ^b	9 ± 0.4 ^c	7 ± 0.6 ^{c*}	2 ± 0.2 ^c	2 ± 0.3 ^b	4 ± 0.2 ^d	5 ± 0.6 ^c
200	101 ± 2.9 ^b	140 ± 4.8 ^{b*}	20 ± 1.9 ^b	30 ± 5.7 ^{ab}	20 ± 0.8 ^b	16 ± 0.6 ^{b*}	4 ± 0.4 ^{bc}	2 ± 0.3 ^{ab*}	9 ± 0.5 ^c	8 ± 0.3 ^b
300	154 ± 5.5 ^a	203 ± 4.1 ^{a*}	34 ± 2.8 ^a	32 ± 1.6 ^{ba}	32 ± 1.9 ^a	20 ± 1.4 ^{b*}	6 ± 0.5 ^{ab}	4 ± 0.2 ^{ab*}	13 ± 0.9 ^b	11 ± 0.6 ^a
400	191 ± 5.1 ^{ab}	176 ± 10.2 ^a	45 ± 3.9 ^a	43 ± 3.3 ^a	40 ± 3.2 ^a	28 ± 2.2 ^{a*}	7 ± 0.5 ^a	5 ± 0.9 ^a	16 ± 0.4 ^a	12 ± 0.6 ^{a*}
Regression analysis										
r^2	0.98	0.89	0.93	0.67	0.95	0.92	0.89	0.77	0.97	0.92
Coefficient										
Intercept	16.62†	-42.36	-3.27	11.42†	-1.31	1.13	-0.02	0.18	-2.30	3.69†
Linear (gossypol)	0.44†	1.41†	0.12†	0.08†	0.11†	0.07†	0.02†	0.01†	0.07†	0.02†
Quadratic (gossypol)	—	0.00†	—	—	—	—	—	—	0.00†	—

^{a-c}Values within a column without a common letter superscript differ ($P < 0.05$).

¹Values are means ± SEM per bird with 6 replicate pens of 4 birds at 21 d and 3 replicate pens of 4 birds at 42 d for each diet.

²No gossypol was detected in the tissues of birds fed 0 mg of gossypol/kg of diet.

*Values for a tissue differ significantly between 21 and 42 d of age ($P < 0.05$).

†Regression coefficient value is significant ($P < 0.05$).

TABLE 4. Concentrations at 21 d and 42 d of age of (+)-gossypol in tissues from broilers fed varying levels of gossypol from gossypol acetic acid (experiment 1)

Dietary gossypol	Liver		Heart		Kidney		Muscle		Plasma	
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
	— (µg/g of DM) ^{1,2} —									
100	200 ± 4.7 ^d	303 ± 24.2 ^{c*}	14 ± 1.7 ^d	27 ± 6.6 ^c	32 ± 2.7 ^c	30 ± 1.3 ^d	4 ± 0.7 ^d	5 ± 0.3 ^b	8 ± 0.1 ^d	11 ± 1.3 ^c
200	353 ± 8.8 ^c	554 ± 19.8 ^{b*}	30 ± 1.9 ^c	44 ± 5.4 ^{cb}	61 ± 4.4 ^b	58 ± 2.3 ^c	9 ± 0.5 ^c	8 ± 0.3 ^{ab}	18 ± 1.2 ^c	17 ± 0.5 ^b
300	540 ± 21.7 ^b	828 ± 16.9 ^{a*}	49 ± 2.7 ^b	54 ± 0.4 ^{ba}	94 ± 4.4 ^a	78 ± 4.9 ^b	13 ± 0.6 ^b	11 ± 0.8 ^{ab}	25 ± 1.1 ^b	23 ± 1.6 ^a
400	686 ± 26.0 ^a	827 ± 29.4 ^{a*}	61 ± 1.4 ^a	64 ± 2.5 ^a	114 ± 8.3 ^a	99 ± 4.6 ^a	16 ± 0.3 ^a	15 ± 3.0 ^a	32 ± 0.4 ^a	27 ± 1.4 ^{a*}
Regression analysis										
r^2	0.98	0.96	0.97	0.81	0.94	0.96	0.97	0.73	0.98	0.91
Coefficient										
Intercept	33.05	-148.87	-1.26	17.02†	5.13	9.07	-1.85	1.61	-4.20	6.00†
Linear (gossypol)	1.65†	4.99†	0.16†	0.12†	0.28†	0.23†	0.07†	0.03†	0.13†	0.05†
Quadratic (gossypol)	—	0.00†	—	—	—	—	0.00†	—	0.00†	—

^{a-d}Values within a column without a common letter superscript differ; $P < 0.05$.

¹Values are means ± SEM per bird with 6 replicate pens of 4 birds at 21 d and 3 replicate pens of 4 birds at 42 d for each diet.

²No gossypol was detected in the tissues of birds fed 0 mg of gossypol/kg of diet.

*Values for a tissue differ significantly between 21 and 42 d of age; $P < 0.05$.

†Regression coefficient value is significant; $P < 0.05$.

TABLE 5. Body weight, feed consumption and feed conversion at 21 d of age for birds fed (+)- or (-)-gossypol (experiment 2)

Dietary gossypol (mg/kg)	Body weight (g) ¹	Total feed consumption (g) ¹	Feed conversion (g/g) ¹
0	723 ± 9.0 ^a	935 ± 15.57 ^a	1.39 ± 0.02 ^a
200 (-)	643 ± 13.6 ^b	801 ± 21.04 ^{bc}	1.35 ± 0.02 ^a
400 (-)	512 ± 5.0 ^c	632 ± 25.50 ^c	1.37 ± 0.05 ^a
200 (+)	666 ± 14.4 ^b	845 ± 21.54 ^{ab}	1.38 ± 0.01 ^a
400 (+)	672 ± 5.1 ^b	871 ± 11.61 ^{ab}	1.40 ± 0.01 ^a

^{a-c}Values within a column without a common superscript differ ($P < 0.05$).

¹Values are means ± SEM per bird with 3 replicate pens of 4 birds for each diet.

most detrimental (Table 5). Dietary supplementation with (+)-gossypol did not significantly affect feed consumption, but addition of (-)-gossypol did reduce feed intake of broiler chicks (Table 5). Water consumption adjusted for feed intake was not different among dietary treatments (data not shown). One bird died on d 4 of this experiment and was from the group fed 200 mg of (-)-gossypol/kg of diet.

Weights of testes, heart, and kidney relative to BW were unaffected by dietary gossypol (Table 6). Compared with the control birds, relative liver weight increased ($P < 0.05$) in chicks fed the highest concentration of (+)-gossypol and decreased ($P < 0.05$) in chicks fed the highest level of (-)-gossypol (Table 6).

Liver had the highest accumulation of both gossypol enantiomers followed by bile, spleen, kidney, testes, heart, plasma, and muscle (Table 7). Accumulation of (+)-gossypol was higher ($P < 0.05$) than that of (-)-gossypol in all of the tissues examined except for bile (Table 7). Only the enantiomer that was fed was detected in the tissues.

The amount of bile contained in the gallbladder was greater ($P < 0.05$) in birds fed 200 and 400 mg of (+)-gossypol and 400 mg of (-)-gossypol than in control birds (Table 6). However, bile concentrations (as determined by DM and spectrophotometric analyses) were not different among dietary treatments (data not shown). Packed cell volumes were 35.1 ± 0.8 , 32.2 ± 0.4 , 32.4 ± 0.8 , 32.2 ± 0.4 , and $32.3 \pm 0.7\%$ for birds fed 0, 200 (-), 400 (-), 200 (+), and 400 (+) mg of gossypol/kg of diet, respectively. Birds fed either enantiomer had lower packed cell volumes than birds fed the control diet ($P < 0.05$).

DISCUSSION

The results of the GAA study were consistent with the widely reported idea that dietary gossypol from CSM negatively affects weight gain and feed efficiency of broilers (Lillie and Bird, 1950; Milligan and Bird, 1951; Couch et al., 1955; Heywang and Bird, 1955; Phelps, 1966; Waldroup, 1981). The current results were inconsistent, however, with a previous experiment (Henry et al., 2001) in which similar levels of partially purified gossypol were fed to broilers, but decreases in BW and feed consumption were not noted. Henry et al. (2001) did report, however, significant decreases in BW gain and feed intake when they fed broilers diets containing 800 and 1,600 mg of gossypol/kg of diet.

Joseph et al. (1986) reported that the amount of (-)-gossypol required to produce cytotoxicity in human tumor-derived cells was approximately 10% of that required with (+)-gossypol. Furthermore, Wang and co-workers (1987) reported that (-)-gossypol had an antifertility effect in male rats, whereas (+)-gossypol had neither an antifertility nor a toxicity effect at the same dose. In the present research, (-)-gossypol had a greater detrimental effect on feed consumption and BW gain than (+)-gossypol. Previous work utilizing CSM with a high (+)-to(-) enantiomer ratio also indicated that (-)-gossypol might be more toxic to broilers than (+)-gossypol (Gamboa et al., 1997; Bailey et al., 2000). Therefore, it would be preferable to use a CSM with a high (+)-to(-) enantiomer ratio in broiler diets, as it would be less detrimental to feed intake. Our results indicate, however, that diets containing 400 mg of (+)-gossypol/kg of diet also affect

TABLE 6. Organ weights at 21 d of age for broilers fed (+)- or (-)-gossypol (experiment 2)

Dietary gossypol (mg/kg)	Heart	Liver	Testes	Kidney	Spleen	Bile
	(g/100 g of BW) ¹					(mL/100 g of BW) ¹
0	0.61 ± 0.03	2.35 ± 0.09 ^{bc}	0.03 ± 0.005	0.34 ± 0.01	0.10 ± 0.01 ^{ab}	0.07 ± 0.01 ^b
200 (-)	0.60 ± 0.03	2.21 ± 0.08 ^{bc}	0.02 ± 0.002	0.31 ± 0.02	0.10 ± 0.02 ^{ab}	0.09 ± 0.01 ^{ab}
400 (-)	0.62 ± 0.04	2.07 ± 0.12 ^c	0.02 ± 0.002	0.33 ± 0.03	0.09 ± 0.01 ^b	0.11 ± 0.01 ^a
200 (+)	0.65 ± 0.07	2.50 ± 0.15 ^{ab}	0.02 ± 0.004	0.35 ± 0.04	0.10 ± 0.01 ^{ab}	0.11 ± 0.02 ^a
400 (+)	0.58 ± 0.02	2.73 ± 0.09 ^a	0.02 ± 0.002	0.36 ± 0.02	0.13 ± 0.01 ^a	0.11 ± 0.01 ^a

^{a-c}Values within a column without a common letter superscript differ ($P < 0.05$).

¹Values are means ± SEM per bird with 3 replicate pens of 4 birds for each diet.

TABLE 7. Concentration at 21 d of age of (+)- and (-)-gossypol in tissues from broilers fed the pure gossypol enantiomers (experiment 2)

Dietary gossypol (mg/kg)	Liver	Spleen	(µg/g of DM) ^{1,2,3}				(µg/mL) ^{1,2,3}		
			Kidney	Testes	Heart	Muscle	Plasma	Bile	
200 (-)	354 ± 11 ^{b*}	52 ± 5 ^{b*}	40 ± 3 [*]	43 ± 13 [*]	44 ± 3 ^{b*}	10 ± 1 ^{b*}	17 ± 1 ^{b*}	291 ± 53	
400 (-)	566 ± 58 ^{a*}	113 ± 11 ^{a*}	82 ± 19 [*]	21 ± 12 [*]	70 ± 7 ^{a*}	15 ± 1 ^{a*}	29 ± 2 ^{a*}	377 ± 32	
200 (+)	878 ± 57 ^x	149 ± 9 ^x	152 ± 27	137 ± 24	70 ± 6 ^x	25 ± 1 ^x	36 ± 1 ^x	430 ± 42	
400 (+)	1176 ± 69 ^y	251 ± 16 ^y	210 ± 4	195 ± 21	111 ± 4 ^y	40 ± 0 ^y	55 ± 0 ^y	540 ± 72	

^{a,b}Values for (-)-gossypol for a given tissue without a common superscript differ ($P < 0.05$).

^{x,y}Values for (+)-gossypol for a given tissue without a common superscript differ ($P < 0.05$).

¹Values are means ± SEM per bird with 3 replicate pens of 4 birds for each diet.

²No gossypol was detected in the tissues of birds fed 0 mg of gossypol/kg of diet.

³There was no (-)-gossypol detected in any of the tissues from birds fed (+)-gossypol and there was no (+)-gossypol detected in any of the tissues from birds fed (-)-gossypol.

*Values for (-)-gossypol are different than the corresponding values of (+)-gossypol ($P < 0.05$).

broiler growth. Thus, the development of a strain of cotton with a high (+)-to(-) enantiomer ratio would decrease but not eliminate the negative impacts of gossypol on broilers. Although gossypol plays a role in protecting the cotton plant from pathogens, altering the enantiomeric ratio in cotton plants may not adversely affect the resistance to certain fungal pathogens (Puckhaber et al., 2002).

Fish et al. (1995) reported that gossypol amine condensates racemize quickly when exposed to sunlight. Racemization of gossypol amine has also been observed to occur in the dark, albeit at a slower rate (Si et al., 1990). Because gossypol in animal tissues is likely to be at least partially bound, the potential of racemization was also considered. In the present study, no evidence of racemization was found in any of the tissues tested. This stability suggests that racemization may occur for only specific gossypol amine complexes or that the reaction is inhibited in aqueous environments. The apparent lack of racemization supports the possibility of incorporating CSM with a high (+)-to(-) ratio of gossypol in poultry diets.

In the present research, accumulation of (+)-gossypol was typically twice that of (-)-gossypol in all broiler tissues examined. In lambs fed cottonseed (Kim et al., 1996) and rats fed pure gossypol enantiomers (Chen et al., 1987), some tissues had higher levels of (+)-gossypol than (-)-gossypol, but other tissues had either the reverse situation or equal concentrations of the 2 enantiomers. Chen et al. (1987) determined that (-)-gossypol has a shorter half-life and a higher clearance rate than (+)-gossypol in rats fed pure (+)- or (-)-gossypol. Based on the shorter half-life, Chen and coworkers (1987) speculated that the toxicity observed with the (-)-gossypol might be attributed to its metabolites. Thus, in broilers, the lower tissue concentrations of (-)-gossypol compared with (+)-gossypol may also result from a higher rate of clearance. In addition to possible differences in clearance, lower tissue concentrations of (-)-gossypol in broilers might also be attributed to differences in intestinal absorption or in the rate of conversion to other metabolites between the 2 gossypol enantiomers.

Results of both experiments suggested that when broilers are fed a fixed dietary gossypol concentration, tissue gossypol levels rise until they reach a maximum value.

With the exception of the liver, tissue concentrations of the enantiomers did not continue to accumulate between 21 and 42 d of age, and in some tissues the levels of (-)-gossypol were marginally lower at 42 d than at 21 d. Previous long-term feeding trials with diets containing gossypol given to fish (Roehm et al., 1967), rats (Jensen et al., 1982), and broiler breeder pullets (Lordelo et al., 2004) also indicate that tissue gossypol levels plateau after a few weeks of feeding.

Gossypol accumulation was highest in livers of broilers fed gossypol from GAA. This result agrees with previous reports about fish (Roehm et al., 1967), lambs (Kim et al., 1996), rats (Sharma et al., 1966; Chen et al., 1987), and broilers (Gamboa et al., 2001). Feeding diets containing gossypol also resulted in increased liver size in broilers (Henry et al., 2001), lambs (Morgan et al., 1988; Kandyliis et al., 1998), and rats (Jensen et al., 1982). In the case of broilers, the current results indicate that the increased liver weight observed when feeding gossypol was due mostly to the accumulation of (+)-gossypol and not (-)-gossypol. Finally, bile production was greater in broilers fed either gossypol enantiomer, which probably indicates that both enantiomers are eliminated from the liver via bile. Sharma et al. (1966) also suggested that bile served as an important excretion route for gossypol in pigs.

The lower packed red blood cell volume in birds fed both enantiomers was not unexpected because dietary gossypol has been found to reduce iron absorption and its bioavailability for hemoglobin formation in several animal species (Herman and Smith, 1973; Clawson et al., 1975; Berardi and Goldblatt, 1980).

In summary, the present studies indicate that both enantiomers of gossypol could adversely affect performance in broilers; however, toxicity was considerably more severe with (-)-gossypol due to its detrimental impact on feed intake. Unlike some species, (+)-gossypol accumulates in broiler tissues to a greater extent than (-)-gossypol, suggesting that (-)-gossypol may be cleared from the body more quickly, absorbed from the intestine at a lower rate, or more rapidly converted to a metabolite than (+)-gossypol. Finally, the results suggest that despite some small antinutritive effects associated with the (+)-enantiomer, CSM with a high ratio of (+)- to (-)-gossypol might

be a suitable feed ingredient for efficient broiler production.

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